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RESEARCH ARTICLE

Escaping the flow: boundary layer use by the darter *Etheostoma tetrazonum* (Percidae) during benthic station holding

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SUMMARY

Aquatic habitats characterized by directional water flow (lotic environments) pose numerous challenges to their inhabitants, including the constant threat of dislodgement and downstream transport. As a result, many organisms exhibit morphological and/or behavioral adaptations that facilitate midwater or benthic station holding in these environments, such as the ventral sucker disc of armored catfishes. However, a few groups, including the species-rich group of small (7–8 cm long and 1–2 cm high) North American stream fishes called darters, exhibit no obvious morphological adaptations to life in lotic habitats. We therefore asked whether small size itself facilitates benthic station holding in these fish. We first used digital particle image velocimetry to quantify the fluid dynamics of flow over a variety of substrates. We then visualized the patterns of flow over the darter *Etheostoma tetrazonum* during benthic station holding. The thickness of the region of decreased water velocity (i.e. the boundary layer) associated with several types of rocky substrate was large enough (~2 cm high in some cases) for *E. tetrazonum* and many other darter species to escape the oncoming flow. We also found that, despite the large size of its pectoral fins, *E. tetrazonum* is capable of producing only very weak negative lift forces with fins. These substrate-directed forces likely act in conjunction with upstream-directed frictional forces between the tail, anal and pelvic fins and the substrate to facilitate station holding. Thus, we hypothesize that, in darters, small size is an adaptation to life in the benthic boundary layer of lotic environments.

Key words: DPIV, digital particle image velocimetry, fish, fluid dynamics, pectoral fin, substrate, vorticity.

INTRODUCTION

Lotic, or flowing water, environments such as creeks, streams and rivers are characterized by measurable directional flow (Allan, 1995; Hart and Finelli, 1999). In many temperate and tropical regions, these habitats often also exhibit high biodiversity (Pearson and Boyero, 2009). This combination of high organismal diversity and a rigorous physical environment is particularly interesting because life in flowing water is fraught with challenges: flowing water has the potential to strongly reduce organismal fitness by displacing midwater and substrate-associated species away from known food sources, mates and desirable habitat (Hart and Finelli, 1999; Lancaster and Hildrew, 1993; Power et al., 1988).

To contend with these displacement-inducing forces, many lotic species have evolved behavioral mechanisms and/or morphological adaptations to either stay in place (hold station) in the face of flow or avoid it entirely (Blake, 2006; Statzner and Holm, 1982; Statzner and Holm, 1989; Webb, 1989; Wilga and Lauder, 2001). Invertebrates such as mayfly larvae (Ephemeroptera) have dorso-ventrally flattened bodies, hook-like appendages that grip the substrate and very thin bodies (on the order of 1–2 mm in depth) that allow species to avoid the flow by living completely within the so-called 'boundary layer', i.e. the surface–fluid interface (Brooks et al., 2005; Dodds and Hisau, 1924). Vertebrate species that live on or near the substrate in these habitats, including many fishes, have been shown to orient their bodies and appendages in a manner that helps to prevent downstream slippage (Arnold and Weihs, 1978; Carlson and Lauder, 2010; Webb, 1989; Webb et al., 1996). In addition, some of these species may be small enough to take advantage of substrate-associated reduced flows such as those in the boundary layer or behind and among large rocks and boulders. However, at present, our understanding of the spatial and temporal flow dynamics near complex rocky substrates is insufficient to predict either the height of these regions of relatively low velocity or the optimal body size of organisms that seek to take advantage of these regions.

Flow of a liquid over any surface includes an area of reduced velocity just above the surface–fluid interface called the 'boundary layer'. The size of the boundary layer is known as its height or thickness. When water flows at a measurable rate (i.e. $U \gg 0$) over a stationary substrate, the benthic boundary layer is defined as the region of reduced flow between the surface of the substrate (where $U_x=0$) and the location at which the local speed is 95 or 99% of the free-stream flow (where $U_x=0.95U$ or $0.99U$, respectively) (Anderson et al., 2001; Schlichting, 1979; Silvester and Sleigh, 1985; Vogel, 1994).

Flow speed in close proximity to the substrate is reduced as a result of the no-slip condition and water's viscosity. Water molecules do not slide smoothly at a constant speed over the substrate; instead, they are slowed down because of frictional forces. Depending on the nature of the substrate, the reduction in flow speed below that of the free-stream flow extends over a greater or lesser vertical distance. For example, at the same speed, flow over a flat, smooth substrate will have a thinner boundary layer than that over a topologically complex substrate such as rocks (Hoerner, 1965). Thus, if at low to

moderate flow velocities the boundary layer over a smooth engineered surface is between 1 and 2 mm in height, that over a rough surface will be considerably higher. The thickness of the boundary layer also varies with flow speed. All else being equal, the boundary layer is thinner at higher flow speeds (i.e. at higher Reynolds numbers) than it is at lower speeds (Denny, 1993; Schlichting, 1979).

Several of the most diverse radiations of fishes, including sculpins (Cottidae; 300 species), suckermouth armored catfishes (Loricariidae; 550 species), blennies (Blenniidae; 345 species), gobies (Gobiidae; 1875 species) and darters (Percidae; 240 species), include a large proportion of benthic species that inhabit physically challenging high-gradient freshwater or wave-swept marine environments (Helfman et al., 1999; Nelson, 1994). Most and sometimes all of the species in these lineages have lost the swimbladder and are therefore unable to attain neutral buoyancy (McCune and Carlson, 2004). In the case of the armored catfishes, success in these environments is linked to morphological adaptation: fishes use ventral (modified from the pelvic fins) and oral sucker discs to anchor themselves in torrential flows (MacDonnell and Blake, 1990).

Darters, a species-rich North American radiation of small benthic and hyperbenthic freshwater fishes, exhibit no such obvious morphological adaptations to life in high-flow environments other than enlarged pectoral fins in a few species (Page and Swofford, 1984). However, most species of darters occupy stream and river habitats with measurable water flow. Some species, including the *Nothonotus* darters and *Etheostoma* darters such as *E. swannanoa*, *E. tetrazonum* and *E. podostomone*, even inhabit torrential riffles characterized by extremely high water velocities, e.g. up to 5 m s^{-1} (Carlson, 2008; Page, 1983).

Here, we asked whether small size facilitates benthic station holding in darters by allowing them to escape the brunt of the oncoming flow, therefore representing an adaptation to lotic habitats. To address this question, we first measured the height of the boundary layer and quantified other patterns of downstream flow reduction on several substrates that mimic natural stream environments. In doing so, we provide a set of quantified spatial and temporal velocity data of flows over ecologically relevant stream and riverine substrates. Then, we gathered data on the pattern and magnitude of the flow disturbance induced by a fish's body and pectoral fins in order to better understand the hydrodynamic consequences of benthic station holding in darters. Finally, we discuss whether darters are sufficiently small in body size to occupy the benthic boundary layer or other regions of substrate-associated reduced flow characteristic of streams and rivers in North America.

MATERIALS AND METHODS

Animals

Adult Missouri saddled darters, *Etheostoma tetrazonum* (Hubbs and Black 1940) (Fig. 1A), were collected in August 2008 from the Gasconade River, Pulaski Co., MO, USA, and shipped live to Harvard University, MA, USA. Fish were maintained in groups of five or six in 20 liter aquaria at 19±1°C according to Harvard University IACUC protocols and fed live blackworms once per day. Flow speed and substrate size in the aquaria were adjusted to approximate natural conditions. We used a total of four individuals (standard length=63.7±2.4 mm) in our experiments with live fish.

Digital particle image velocimetry

Experiments were conducted in a calibrated flow tank chilled to 19±1°C (for details, see Tytell and Lauder, 2004). We used digital particle image velocimetry (DPIV) to visualize water flow over various substrates and over the fish during benthic station holding.

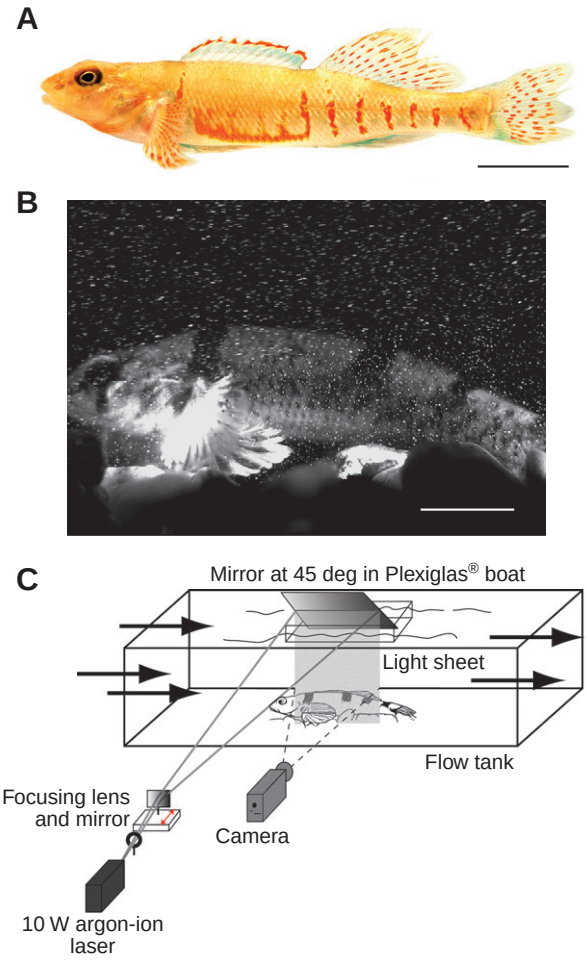

Fig. 1. Study species, an example frame from a high-speed video sequence of a station-holding darter, and experimental setup. (A) A male Missouri saddled darter, *Etheostoma tetrazonum*, from the Gasconade River near Devil's Elbow, Pulaski Co., MO, USA. Scale bar, 1 cm. (B) *E. tetrazonum* holding station on rock substrate in flow at 24 cm s^{-1}. Scale bar, 1 cm. Water is seeded with near-neutrally buoyant particles for digital particle image velocimetry (DPIV); particles in the plane of the vertical laser sheet are illuminated. The laser sheet transects the pectoral and pelvic fins in this sequence. (C) Experimental setup used for DPIV consists of a 600 l variable speed recirculating flow tank seeded with small (12 mm diameter) silver-coated beads. The experimental fish is restricted to a small region in the center of the flow tank that contains substrate. A 10 W argon laser sheet is generated using a series of simple optics and mirrors and oriented vertically by reflection of the sheet off of a front-surface mirror angled at 45 deg and suspended above a Plexiglas® boat. The light sheet thus comes from above. Moving the focusing mirror in the direction indicated by the red arrow alters the location at which the laser sheet transects the fish and/or substrate. Video sequence data were collected by a high-speed video camera recording at 500 Hz with 1024×1024 pixel resolution per image.

The water in the flow tank was seeded with silver-coated near-neutrally buoyant glass beads (mean diameter=12 mm) that reflect laser light (Fig. 1B,C), as previously described (Drucker and Lauder, 1999; Standen and Lauder, 2007; Wilga and Lauder, 2001). Substrates and fish were filmed in lateral view using a single Photron Fastcam high-speed video camera (1024×1024 pixel resolution; San Diego, CA, USA) recording at 500 frames s^{-1} (Fig. 1C). We used a series of simple optics and lenses to focus a 10 W argon-ion laser into a thin light sheet. We reflected the light sheet off of a front surface mirror angled at 45 deg and positioned above the flow tank

in a Plexiglas® boat to create a vertical light sheet (1–2 mm×15 cm, depth×width). The boat served to eliminate movement of the water surface. By moving one of the mirrors forwards and backwards along a track (red arrow, Fig. 1C), we were able to change the position of the vertical light sheet and the location at which it transected the station-holding fish. In this way, we could visualize patterns of flow at multiple locations along the fish's body and pectoral fin without forcing it to alter its posture.

We gathered video sequence data of water flow over four types of substrates without a station-holding fish and over two types of substrate with a fish. To visualize patterns of water flow over substrates without a fish, we filmed flow over smooth Plexiglas® at 0, 11 and 24 cm s^{-1}, and over small rocks (mean diameter=10 mm), over small rocks arranged to form a deep depression in the center (dip) and over a large, flat rock (70×51 mm) on top of the small rocks (rocks + big rock) at 0, 4, 17 and 31 cm s^{-1}. The rock substrates were arranged in a small tray to prevent them from being swept downstream. Because the boundary layer associated with flow over a flat plate will grow and approach final thickness at points downstream of the leading edge of the plate (Hoerner, 1965; Prandtl and Tietjens, 1934; Schlichting, 1979), we ensured that flow over our substrate tray was stable and that the boundary layer did not grow within the region of which images were captured. Additionally, we note that Reynolds numbers of the flows over our substrates (using the measured boundary layer thickness as the length scale; see below) ranged from 1000 to 4000. To visualize patterns of water flow over substrates with a fish, we filmed four darters station holding on the small rock substrate at speeds from 0 to 31 cm s^{-1} and on Plexiglas® at speeds from 8 to 37 cm s^{-1}.

Substrate-related flow visualization and quantification

To compare patterns of water flow over the four substrate types, we digitized sequential pairs of N frames (2 ms apart) from the video sequences using standard two-frame cross-correlation analysis in the program DaVis (version 7.2, LaVision Software, Inc., Goettingen, Germany). This analysis yielded a matrix of ~17,000 vectors (132×132 vectors) for each of N–1 frames, as in previous research (Lauder and Madden, 2008; Standen and Lauder, 2007; Tytell and Lauder, 2004). We used the resulting data to quantify various aspects of water flow over the substrate as described below.

To our knowledge, few previous studies have quantified patterns of substrate-associated flow using techniques such as DPIV with the goal of generating a spatial velocity map over a variety of highly textured substrates typical of natural lotic habitats. Such measurements allow determination of boundary layer thickness relative to the size of organisms living in the boundary layer region as well as provide an estimate of the extent of flow variation through time. Some previous work has measured stream flow characteristics in the field (Cotel et al., 2006) and boundary layer profiles over beds of varying roughness [Rahman and Webster (Rahman and Webster, 2005) and references therein], but both temporal and spatial, but flow profiles in the near-bottom region over substrates of differing complexity and roughness relevant to benthic fishes are not available. Thus, in order to provide a quantitative description of the flow environment over a variety of stream and riverine substrates, we estimated a series of standard variables from high-speed video of water flow over each of the four substrate types at moderate speeds: 11 cm s^{-1} for Plexiglas® and 17 cm s^{-1} for rocks, dip and rocks + big rock. From representative frames of each of the four resulting sequences, we calculated flow velocity (x-direction; V_x), strain rate (E_{xy}) and vorticity (z-axis rotation). The sign associated with vorticity indicates the direction of rotation; here, a negative sign indicates counterclockwise rotation whereas a positive sign indicates clockwise rotation. In combination, strain rate and vorticity describe the magnitude and direction, respectively, of two of the primary stresses associated with flowing water that are relevant to small benthic fishes. In addition to these metrics, we calculated the root mean square (r.m.s.) of V_x over the duration of the sequence in order to determine the extent of spatial and temporal variation in flow speed and direction.

From these data, we generated V_x and r.m.s.-V_x profiles to compare the height (distance above the substrate) and temporal variability in the boundary layer or substrate-associated region of reduced flow, as appropriate, among the four substrate types. A velocity profile is a plot of height *versus* local speed. Similarly, an r.m.s.-V_x profile is a plot of height *versus* r.m.s.-V_x. Our profiles were generated by extracting data on flow speed and direction along a single vertical transect from a grid of vector means. Free-stream flow speed was 17 cm s^{-1} over the rocks, dip and rocks + big rock substrates and 11 cm s^{-1} over Plexiglas®. We chose to generate velocity and r.m.s.-V_x profiles of flow over Plexiglas® at a slightly lower speed than flow over the three rock substrates because the Plexiglas® boundary layer is extremely thin even at low speeds. Mean V_x and r.m.s.-V_x were calculated from sequences of variable length: 400 (rocks and dip), 600 (rocks + big rock) or 1000 (Plexiglas®) frames. Transects were taken from the approximate center of the rock and Plexiglas® frames, in the center of the dip and ~2 cm behind (i.e. downstream of) the big rock.

From the velocity profiles, we used the piecewise regression function in SigmaPlot (version 11.2, Systat Software, Inc., Chicago, IL, USA) to determine the height of the boundary layer or substrate-associated region of reduced flow (as appropriate) and the magnitude of the velocity gradient. We used the regressions to identify the point of transition (TP) between the substrate (V_x=0 cm s^{-1}) and the free-stream flow. We then determined the location of the TP and estimated the height of the boundary layer (or substrate-associated region of reduced flow) as 0.99 TP. We calculated the magnitude of the flow gradient as the slope of the line between the substrate (or substrate-associated flow) and the TP. The flow gradient is therefore equal to the change in height per unit change in velocity or, put another way, the derivative of height with respective to velocity. From the r.m.s.-V_x profiles, we estimated the height above the substrate with the greatest temporal variation in flow velocity.

We also generated velocity and r.m.s.-V_x profiles of water flow over rocks at two additional speeds, 4 and 31 cm s^{-1}, in order to determine the extent to which boundary layer height changes with increasing flow speed. Again, we used the profiles to estimate, at each speed, the height of the boundary layer and the magnitude of the flow gradient as well as the height with the greatest temporal variation in flow speed and direction (r.m.s.-V_x).

Fish-generated flows

Small benthic fishes will obstruct and disturb the downstream flow of water in a manner similar to that of the large flat rock used in our rocks + big rock substrate. In contrast to a rock, however, the fish's median and paired fins may disrupt the downstream flow in an organized manner that facilitates station holding. We therefore recorded video data of *E. tetrazonum* station holding on the rock and Plexiglas® substrates at a variety of speeds (see above) in order to quantify fish-generated patterns of flow disruption and circulation.

Fish were added to the flow tank (set at a low speed) and allowed to acclimate for ~30 min prior to filming. Individuals were then gently coaxed into the approximate center of the substrate

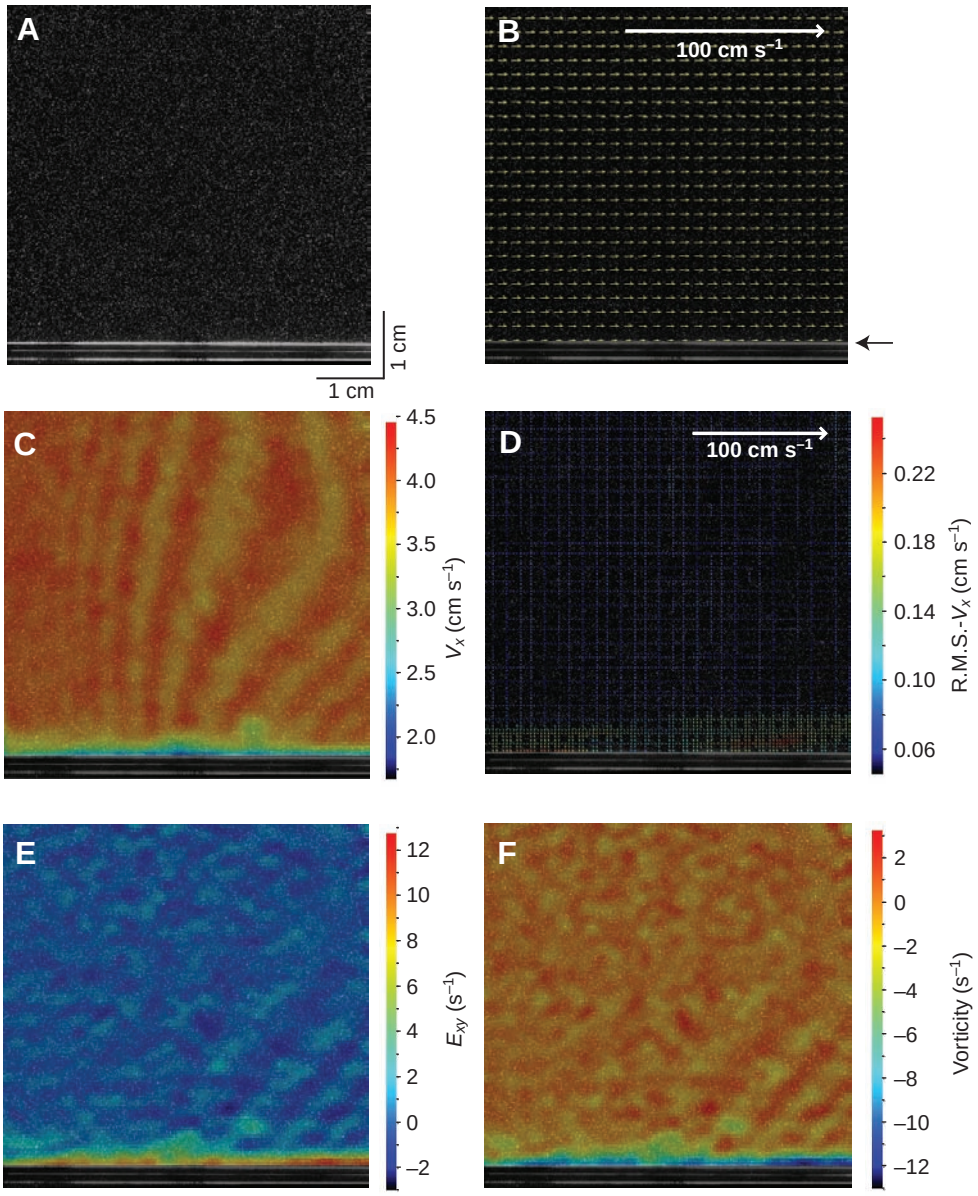

Fig. 2. Basic characteristics of flow over Plexiglas at 11 cm s^{-1}. (A) Representative frame of the high-speed video sequence from which patterns of flow were quantified. (B) Flow velocity (*x*-direction; V_x). Every fourth vector is shown. Orientation and length of yellow arrows indicate the speed and direction of water flow. Black arrow on the right side of the panel indicates the surface of the Plexiglas. (C) Velocity (*x*-direction) of water flow illustrated by a color gradient. The region of reduced flow velocity near the substrate is clearly visible (dark to light blue; ~1–2 mm thick, see text). Temporal variation in V_x is also visible as alternating red and orange bands indicating relatively faster and slow flow. (D) Temporal variability in flow velocity estimated as root mean square (r.m.s.)-V_x from a sequence of 1000 frames (2 s). Both arrow length and color indicate the magnitude of variation in speed whereas arrow orientation indicates the direction of variation. Areas of high r.m.s.-V_x, such as near the substrate, are indicated by red and orange arrows. (E) Strain rate (E_{xy}) of flow illustrated by a color gradient, with high strain rates visible near the substrate. (F) Vorticity (*z*-rotation) of flows illustrated by a color gradient. Sign indicates the direction of rotation: negative is counterclockwise, positive is clockwise. Color indicates the magnitude of rotation, i.e. rotational speed. All panels except D show the same frame; the r.m.s. analysis (D) includes this frame.

tray and given time to take on a station-holding posture. If the flow speed was sufficiently low, fish were content to hold position for an extended period of time. In these cases, we were able to gather video sequences of flow at various locations along the fish by moving only the laser sheet as described above (see also Fig. 1C). Following video sequence acquisition, we used DaVis software to visualize patterns of particle flow over and around the body and pectoral fins of the station-holding darter as described above. We also used the video sequences to estimate the magnitude of the substrate-directed (i.e. negative lift) forces generated by the organized circulation behind the pectoral fins by estimating the downward force from the regularly shed vortices, as previously described (Drucker and Lauder, 1999; Standen and Lauder, 2007; Wilga and Lauder, 2001).

Station-holding kinematics

In our previous paper (Carlson and Lauder, 2010), we compared station-holding posture between two species of *Etheostoma* darters using short video sequences of flow across a range of speeds. Here, we instead gathered long DPIV video sequences (i.e. 1000–3000 frames in length or 2–6 s in duration) of *E. tetrazonum* holding station on rocks and Plexiglas® across a range of speeds from near 0 to 38 cm s^{-1}. We then visualized patterns of water flow during these sequences using DaVis software as above. We also gathered kinematic data from the video sequences in order to determine how fin orientation changes with increasing speed and whether the pattern of change differs between fish holding station on Plexiglas® and rock substrates. First, because changes in fin position were slight, we subsampled the video sequences every 200 frames. We then digitized five points on each frame using the DLTv3 program for MATLAB (Hedrick, 2008). Three of the five points were located on the pectoral fin – at the top, middle, and bottom of the vertical section illuminated by the laser – a fourth was located in the center of the fish's eye and a fifth at the intersection of two stationary rocks. We then used data on the location of these points to calculate velocity-related changes in fin position including the angle of the pectoral fin relative to the horizontal, its projected area into the flow and its 'cupping' angle

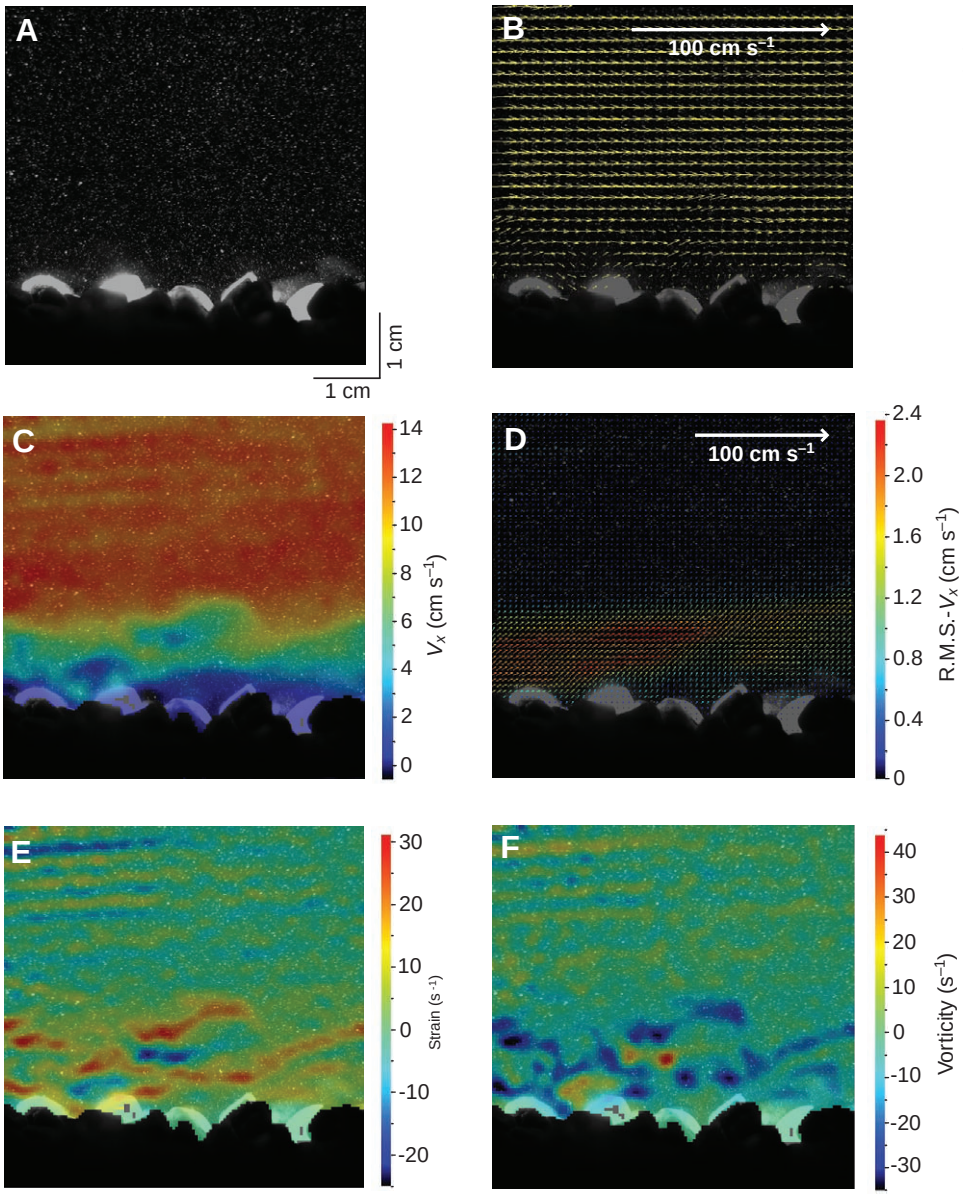

Fig. 3. Basic characteristics of water flow over small rocks at 17 cm s^{-1}. Panels are as described in Fig. 2 except in the case of panel D. Here, r.m.s.-V_x is calculated from a sequence of 400 frames (0.8 s). Note that the height of the boundary layer is greater here (C) than it is on Plexiglas® (Fig. 2C).

(Fig. 12A). This last metric quantifies the extent to which the fin bends outward (i.e. 'cups' into the flow) in response to changes in flow speed. Higher values indicate less cupping. Finally, we used linear regression to determine whether change in any or all of the metrics was significantly correlated with flow velocity. Values are presented as means ± s.e.m.

RESULTS

Patterns of substrate-associated flow

Flow over each of the four substrates – Plexiglas®, rocks, dip and rocks + big rock – was altered in a distinct way by interactions with the unique characteristics of each substrate type. The smooth Plexiglas® caused very little alteration to downstream flow speed and direction (Fig. 2). The boundary layer was thin (a few millimeters high) (Fig. 2B,C, Fig. 6A) and temporal variation in velocity was concentrated near the substrate. Values of r.m.s.-V_x reached a maximum of 0.22 cm s^{-1} ~2 mm above the substrate (Fig. 2D). Strain rate and vorticity were also greatest just above the substrate and reached maxima of 12 and –12 s^{-1}, respectively (Fig. 2E,F).

The flow was considerably more disturbed by rock substrates than it was by Plexiglas®. The boundary layer associated with rock substrate was nearly 2 cm in height (Fig. 3B,C) and included a large and distinct transition region between the near-zero substrate-associated flow and the free-stream flow (shown in green-yellow in Fig. 3C; also compare Plexiglas® with rocks in Fig. 6A). Flow within the boundary layer was always positive (i.e. downstream), except in two small regions just above the substrate (dark blue in Fig. 3C). The region of greatest temporal variation in flow velocity was nearly equal in size to that of the boundary layer (Fig. 3D). The magnitude of temporal variation was ~10 times greater than that on Plexiglas® (2.4 cm s^{-1}), even though the free-stream flow was only 3.1 times faster over the rocks.

Flow over rocks was also characterized by measurable stresses that changed in a non-linear manner with increasing height above the substrate (Fig. 3E,F). Strain rate and vorticity were greatest in the boundary layer. Strain rate was primarily positive in this region, with a few small negative pockets (Fig. 3E). The pattern of fluid vorticity was the inverse of that of strain rate (Fig. 3F); most vorticity

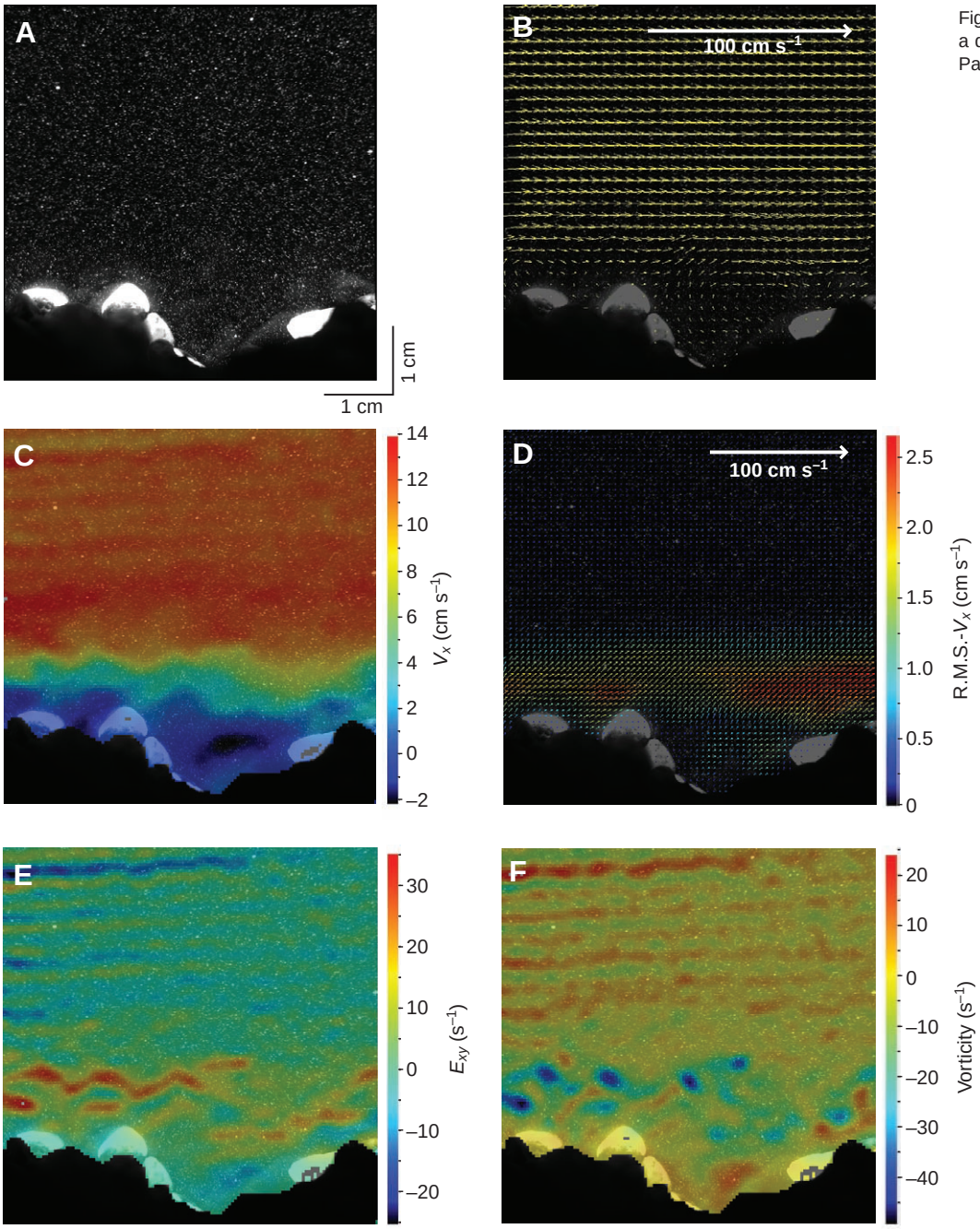

Fig. 4. Basic characteristics of flow over a dip in the substrate at 17 cm s^{-1}. Panels are as described in Fig. 3.

was counterclockwise, but in a few areas it was positive and rapid (up to 40 s^{-1}).

Increasing the flow of water over the rocks had less of an effect on the height of the boundary layer than expected (Fig. 7). At 4 cm s^{-1}, the boundary layer was 1.5±0.008 cm high. At 17 and 31 cm s^{-1}, it was 1.8±0.004 and 1.5±0.006 cm high, respectively. In contrast to boundary layer height, increasing flow speed had a strong effect on both the magnitude of the velocity gradient and the extent of temporal variability in velocity. The velocity gradient was steeper at 31 cm s^{-1} (14.6 s^{-1}) than at 17 cm s^{-1} (8.3 s^{-1}) or 4 cm s^{-1} (2.1 s^{-1}). Likewise, r.m.s.-V_x was greater at 31 cm s^{-1} than at 17 cm s^{-1}, and was lowest at 4 cm s^{-1}.

Patterns of flow alteration due to the dip were generally similar to those due to the rocks, with a few notable exceptions (Figs 3, 4, Fig. 6A). First, the region of reduced flow associated with the substrate (technically not a boundary layer because the alteration in flow was due to a substrate formation and not to the substrate itself) was thicker than the rock boundary layer (Fig. 4B,C). From the bottom of the dip up to the free-stream flow, the region of reduced flow measured ~2.5 cm in height. In addition, the transition zone from near-zero flows (both upstream and downstream in direction) to the free-stream flow was ~1 cm in height or twice that of the same region associated with flow over rocks (Fig. 4C). Finally, there was a distinct pocket of clockwise recirculation in the center of the dip that was larger than that associated with flow over rocks (Fig. 4C,F). It is notable that this recirculation was more constant in magnitude and direction than was flow over the rocks on either side of the dip (Fig. 4D). Overall, however, the maximum extent of temporal variation was similar to that of flow over rocks (maximum r.m.s.-V_x=2.6 cm s^{-1}).

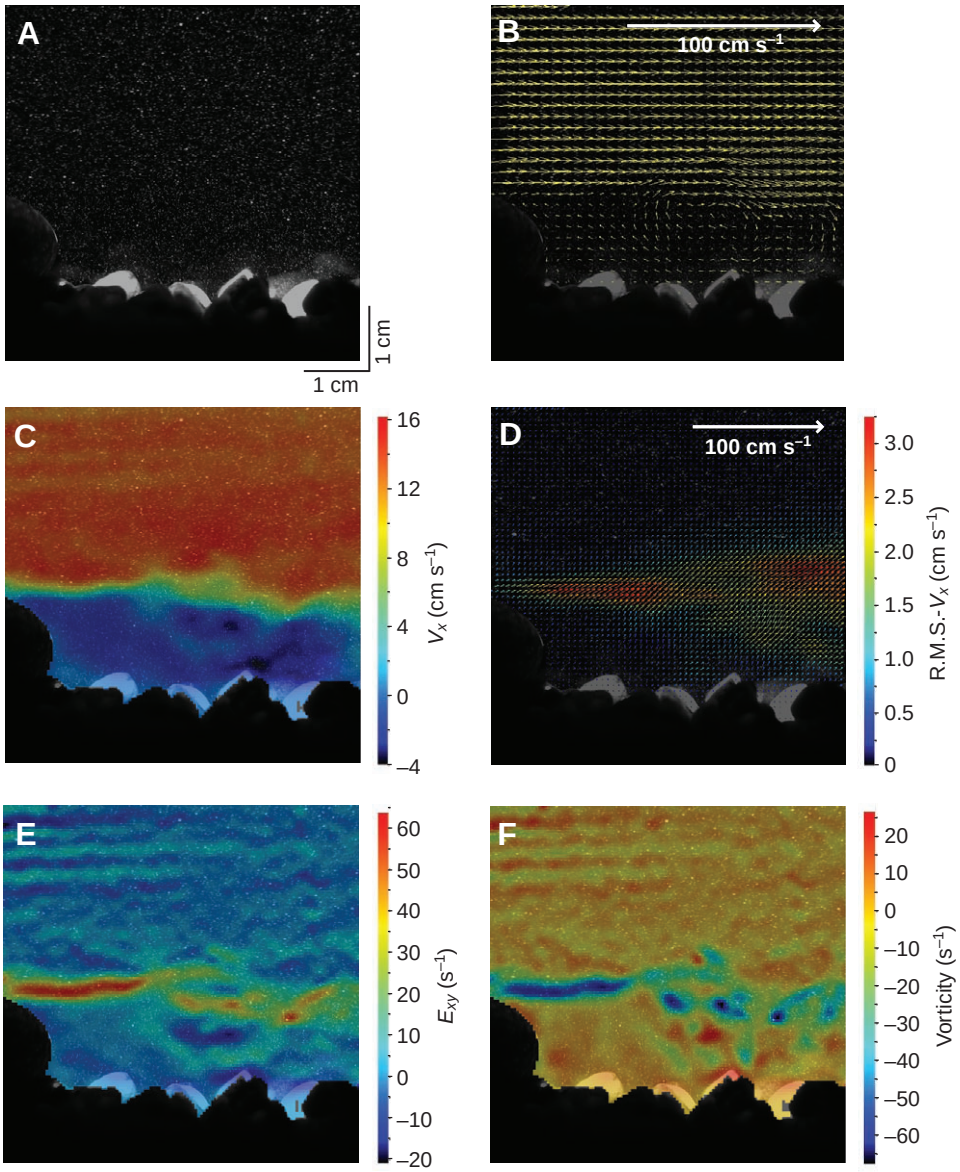

Fig. 5. Basic characteristics of flow over rocks + big rock at 17 cm s^{-1}. Panels are as described in Fig. 2 except in the case of panel D. Here, r.m.s.-V_x is calculated from a sequence of 600 frames (1.2 s).

With the exception of the region of recirculation in the dip, most of the non-linear stresses were associated with flow over the rocks on either side of the dip (Fig. 4E,F). Flow over these rocks produced strain rates equal to ~20–30 s^{-1} and frequent pockets of strong counterclockwise rotation (vorticity=–40 s^{-1}). The strain and rotation induced by the rocks on either side of the dip also altered the free-stream flow and produced distinct bands of negative strain and clockwise rotation.

The presence of a big rock altered downstream flow patterns in a manner that differed from that of either a dip or many small rocks. Specifically, flow over the large flat rock resulted in flow separation and orderly shedding of clockwise-rotating vortices into the downstream flow (Fig. 5B,C,F). Flow separation downstream of the rock was also characterized by high strain rate, on the order of 60 s^{-1}, and rapid counterclockwise rotation (vorticity>–60 s^{-1}; Fig. 5E,F). In addition, the big rock blocked downstream flow, producing a region of near-zero flow just behind it. The region of reduced flow was approximately equal in height to that of the rock (~1.5 cm; Fig. 5C). Temporal variation in velocity was also greatest just behind the big rock (Fig. 5D). From the downstream end of the rock, the region of measurable temporal variation expanded like a cone from 0.5 to 2.5 cm in height.

The velocity profiles confirm that the boundary layer or region of substrate-associated reduced flow (as appropriate) differed in height among the four substrates (Fig. 6). On the substrates containing rocks of one or more sizes, this region ranged in size from 2.4±0.005 cm in the center of the dip, to 2.0±0.002 cm behind the big rock and 1.9±0.004 on rocks only. Even at a relatively lower speed, chosen to aid in visualization, the boundary layer measured only 0.02±0.005 cm on Plexiglas®.

In the case of both the dip and the rocks + big rock, the flow reversed direction (i.e. V_x<0) just above the substrate. Such a reversal was the result of the pattern of counterclockwise circulation in the center of the dip (Fig. 4C) and behind the big rock (Fig. 5C) described above. The flow gradient was greatest over rocks + big rock (22 s^{-1}), then rocks and the dip (8.4 and 8.2 s^{-1}, respectively) and, finally, Plexiglas® (7.5 s^{-1}; Fig. 6).

Flow over the three rock substrates exhibited much greater temporal variation in velocity than did flow over Plexiglas® (Fig. 6). Variation was greater over the rocks + big rock substrate (2.7 cm s^{-1})

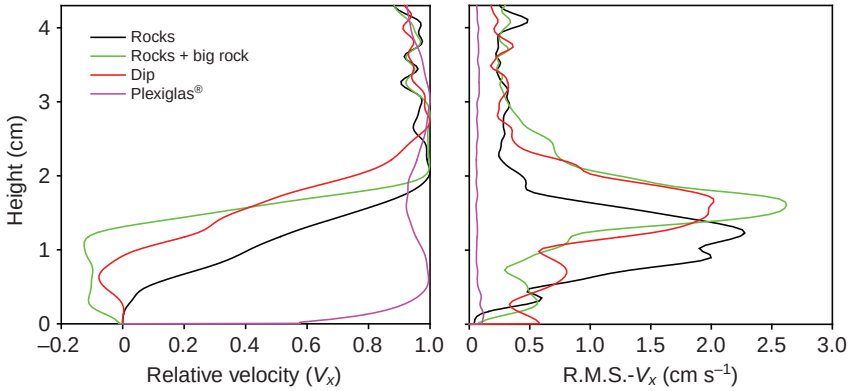

Fig. 6. Height and variability of the Plexiglas® and rock-associated boundary layers and other regions of reduced flow resulting from water movement over each of four substrate types: Plexiglas®, rocks, dip and rocks + big rock. (A) Profiles of relative velocity (*x*-direction) *versus* height above the four substrates. Velocity data for each substrate type are scaled to a value of 1.0 to account for differences in the free-stream flow speed at which video sequence data were collected. (B) r.m.s.-V_x profiles *versus* height above the top of the same four substrates. Both velocity and r.m.s.-V_x values were calculated from sequences of 1000 (Plexiglas®), 400 (rocks and dip) or 600 (rocks + big rock) frames. Note that the same sequences also appear in Figs 2–5.

than over rocks (2.25 cm s^{-1}), and was lowest over the dip (2.0 cm s^{-1}). The greatest variation occurred 1.6 cm (rocks + big rock), 1.65 cm (rocks) and 1.4 cm (dip) above the substrate. In the case of the rocks and the dip, this region occurred well within the boundary layer or region of substrate-associated reduced flow. However, in the case of the rocks + big rock, r.m.s.-V_x was greatest near the upper edge of the region of reduced flow.

Darter-related flows

In the most basic sense, darters (and their expanded pectoral fins) altered the flow in a manner similar to that of a large flat rock similar to the type used in our experiments (Fig. 5C, Fig. 8D). Even at low speeds, the pectoral fin of a station-holding *E. tetrazonum* blocked the incoming flow and produced a pocket of reduced flow downstream of the fin (Fig. 8A). As flow speed increased, the region of reduced flow was extended and a gradient between areas of low and high flow speed was generated (Fig. 8B,C, Fig. 9B). At very high speeds (i.e. >22.5 cm s^{-1}; Fig. 8D), the flow separated as it passed over the dorsal edge of the pectoral fin. The flow separation generated a series of paired weak counter-rotating vortices (Fig. 8D, Fig. 10A). These vortices were associated with a substrate-directed force that averaged –0.000125 mN on the rocky substrate and –0.000435 mN on Plexiglas®, or ~1% of the darter's body weight in water.

Patterns of flow both upstream and downstream of the pectoral fin of station-holding darters differed between fish on Plexiglas® and rock substrates (Figs 9, 10). On Plexiglas®, the incoming flow experienced by the pectoral fin was characterized by a steep velocity gradient (slice 1 in Fig. 9A). In addition, the smooth, flat nature of the Plexiglas® surface allowed the ventral surface of the fish's pectoral fin to be in direct contact with the plastic, preventing water from flowing under the fin along the majority of its length. (The pectoral fin tapers towards the base and thus water is able to flow under the fin near the body but not more distally along the fin.) As a result, the effect of the fin on the downstream flow was rather dramatic: flow speed directly behind the fin (slice 2 in Fig. 9A) was essentially zero. The height of this region was approximately equal to that between the dorsal surface of the pectoral fin and the surface of the Plexiglas® but decreased with increasing distance downstream (slice 3 in Fig. 9A).

In contrast to the patterns of flow over and behind the pectoral fin of a darter holding station on Plexiglas®, flow over and behind the fin of a fish on rocks was more complex. First, the incoming flow was reduced by interaction with the rocks and, compared with the incoming flow on Plexiglas®, was characterized by a shallower velocity gradient (compare slice 1 between Fig. 9A and 9B). In addition, the rocks provided an uneven surface on which the fish could rest both its body and fins. For example, in the video sequence used in Fig. 9B and Fig. 10A, the fish rested its fin against the upstream surface of a rock. As a result, water was able to flow under the ventral edge of the fin. This is clearly visible in the lower panel of Fig. 10A. In a manner similar to that of the fin on Plexiglas®, the fin on rocks blocked the flow and reduced the velocity of the flow behind it. However, because of the jet of water flowing under the fin near the fish's body, the velocity profiles in slices 2 and 3 of Fig. 9B are not smooth near the substrate. Instead, there are two 'peaks' of lower velocity: one at the substrate (height=0 cm) and one at the dorsal edge of the fin (height=1.1 cm). Both the flow reduction by the fin and the effect of the under-fin jet were reduced with increasing distance downstream (slice 4 in Fig. 9B).

The shape and structure of the pectoral fin itself was the same between the fish holding station on the two substrates. As a result,

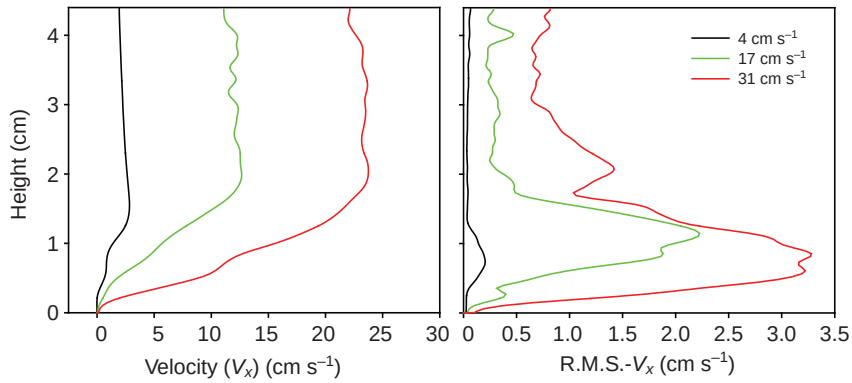

Fig. 7. Height and variability of the boundary layer on rock substrate at three speeds: 4, 17, and 31 cm s^{-1}. All velocity and r.m.s.-V_x profiles were calculated from 400 frames (0.8 s). Note that the sequence of flow at 17 cm s^{-1} is the same as that for rocks in Fig. 6. Estimates of boundary layer thickness values as derived from these plots are given in the text.

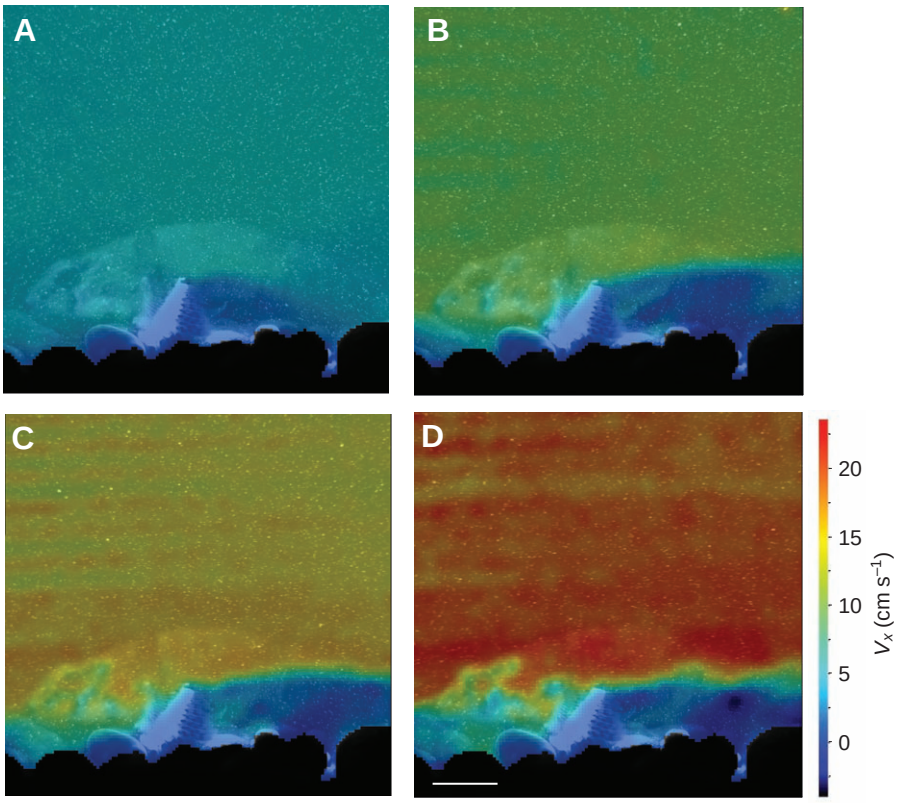

Fig. 8. *E. tetrazonum* holding station at four speeds on rock substrate: (A) 4 cm s^{-1}, (B) 10 cm s^{-1}, (C) 15 cm s^{-1} and (D) 20 cm s^{-1}. In all panels, V_x is illustrated by the same color gradient (to the right of D). Note the change in flow leaving the dorsal edge of the pectoral fin as speed increases. Scale bar in D (applies to all panels), 1 cm.

water flowing downstream over the fin separated and produced a series of counter-rotating vortices when fish were on both Plexiglas® and rocks (Fig. 10A,B).

Downstream patterns of water flow were modified not only by the pectoral fin but also by the body of a station-holding darter (Fig. 11A). The most dramatic effects of the fish's body were: (1) the separation of flows and production of a narrow boundary layer near the surface of the fish and (2) the region of reduced speed and turbulent flows downstream of the fish's second dorsal fin. The height of the region of darter-induced reduced flow was approximately the same behind the head of the fish (slice 1 in Fig. 11B) and behind the second dorsal fin (slice 3 in Fig. 11B).

Pectoral fin kinematics

Station-holding darters exhibited measurable changes in pectoral fin position with increasing flow speed that were qualitatively similar among individuals holding station on rocks and Plexiglas®. As flow speed increased, fish on both substrates lowered their pectoral fins, decreasing the angle between the fin and the substrate (Fig. 12B). At the same time, the fish expanded their pectoral fin rays and increased the cupping angle (Fig. 12C). Lowering of the pectoral fin and increasing the cupping angle combined to decrease the projected area of the fin (Fig. 12D). Changes in all three metrics of fin position were significantly correlated with velocity ($P \leq 0.001$) based on an analysis of two sequences of a darter holding station on rocks.

DISCUSSION

Despite the challenges associated with life in flowing water, a large number of organisms have come to successfully occupy lotic environments (Hart and Finelli, 1999; Lancaster and Hildrew, 1993; Power et al., 1988). Many of these species have evolved some mechanism by which to either remain in place in the face of high flow, through the use of specialized suction cups or grasping hooks, or entirely avoid it by occupying crevices and other refuges in the substrate (Blake, 2006; Dodds and Hisau, 1924; Statzner and Holm, 1982; Webb, 1989). We found that North American darter fish are able to escape much of the oncoming flow by living within the boundary layer or other region of substrate-associated reduced flow as a result of their small size. However, the fish's large and often-expansive pectoral fins generate only relatively weak substrate-directed forces.

Substrate-associated reduced flows and their use by darters

A large body of literature describes the characteristics of the boundary layer region in flowing water. However, almost all of this work has explored the nature of the boundary layer associated with flow over very smooth artificial surfaces such as metal plates. Boundary layers on these surfaces can be very thin, on the order of 1 mm or less (Denny, 1993; Prandtl and Tietjens, 1934; Schlichting, 1979). Similarly, over the surface of a swimming fish, Anderson et al. estimated the height of the boundary layer to be between 1 and 2 mm (Anderson et al., 2001). In contrast, we found that over natural substrates such as our small rocks, the boundary layer was quite thick: on the order of 1 to 2 cm.

We note that the flows observed over the rocky substrates in our experiments are most appropriately termed 'microturbulent'. Furthermore, we did not attempt to fit our boundary layer profiles to either turbulent or laminar flow models (i.e. Denny, 1993; Schlichting, 1979) because no existing theory predicts the laminar/turbulent nature of profiles of flow over uneven rocky substrates such as ours. Instead, we focused on experimentally generating boundary layer profiles of flow over complex substrates like those found in nature. Such data are additionally

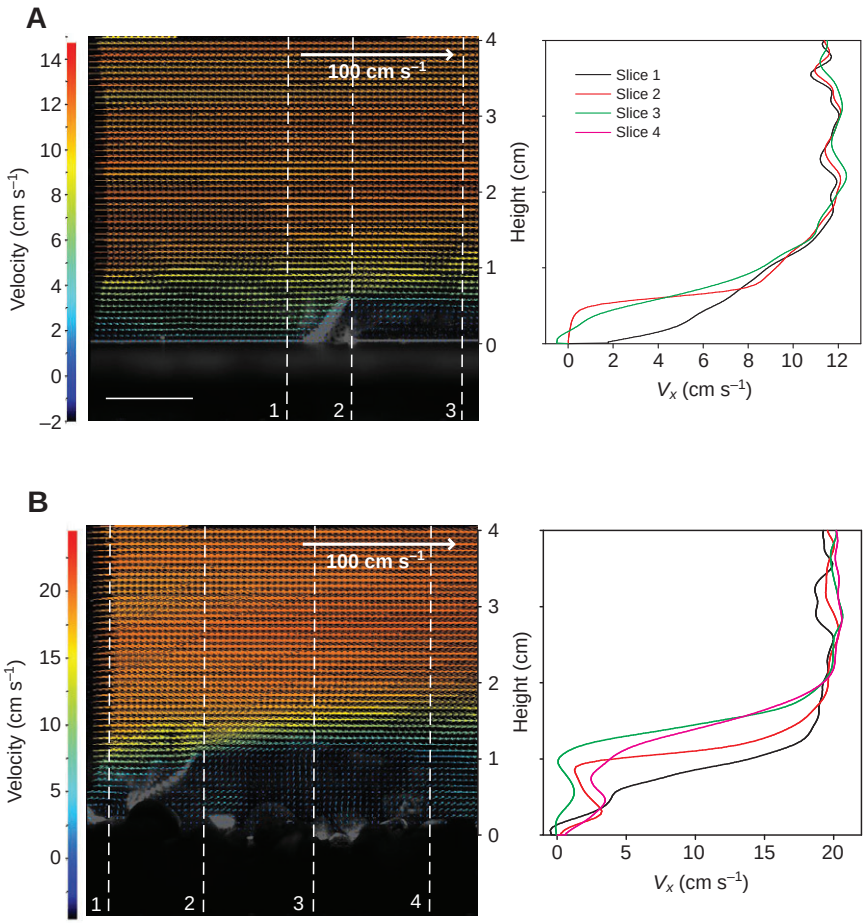

Fig. 9. Velocity profiles from three (A) or four (B) vertical slices in front of and behind the pectoral fin of a station-holding darter on Plexiglas® (A) and rock (B) substrates. Profiles are calculated from 500 (Plexiglas®) or 400 frames (rocks). Note that the free-stream water velocity in the Plexiglas® sequence is approximately half of that in the rock sequence. Scale bar, 1 cm.

important because of the limited data available on spatial and temporal patterns of flow over substrates relevant to benthic fishes, which are not only rough [see Rahman and Webster (Rahman and Webster, 2005) and references therein for data on rough surface profiles] but also contain projecting rocks and depressions which greatly alter free-stream flow. Thus, one general goal of this work is to illustrate the dominant spatial and temporal characteristics of boundary layer flows (e.g. Figs 2–5) and to generate boundary

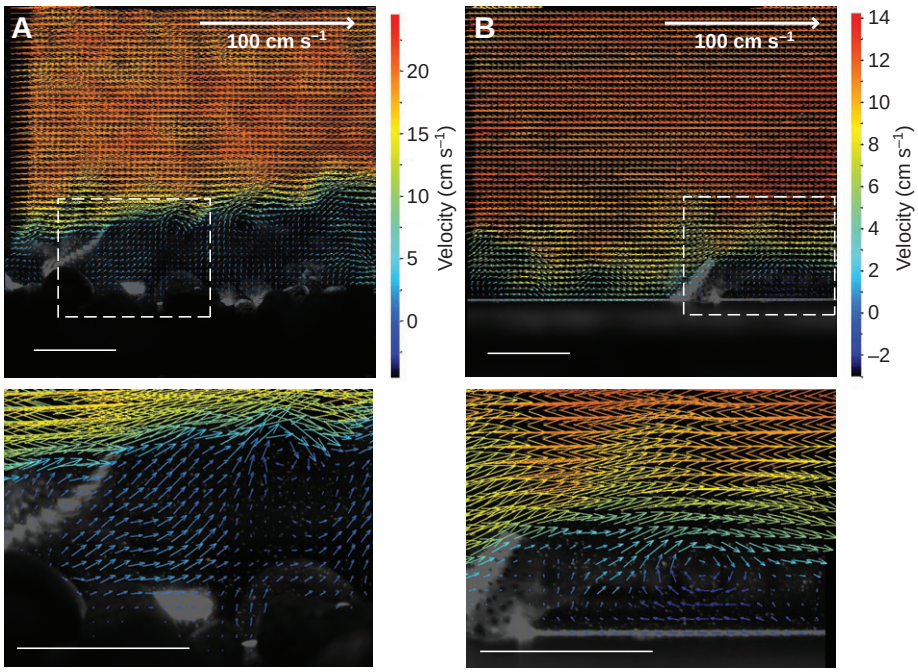

Fig. 10. Representative patterns of vortex formation behind the pectoral fin of *E. tetrazonum* holding station on rocks (A) and Plexiglas® (B). Boxes outlined in white dashed lines correspond to the region enlarged in the two lower panels. Note that arrow length has been doubled in the lower panels for clarity. Scale bars, 1 cm.

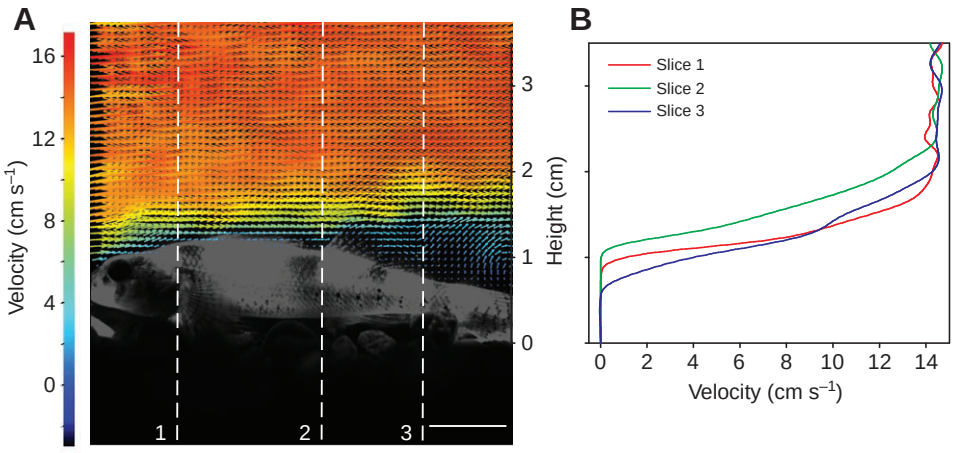

Fig. 11. Darter-induced disturbance to downstream flow. (A) Flow over the dorsal surface of *E. tetrazonum* at 17 cm s^{-1}. Arrow length and color indicate speed; arrow orientation indicates direction of flow. Scale bar, 1 cm. (B) Mean velocity (V_x) profiles at the three slices indicated in A from a sample of 400 frames.

layer or velocity profiles for 'natural' stream and riverine substrates (Figs 6, 7).

Each of the four substrate types that we tested – Plexiglas®, rocks, dip and rocks + big rock – altered the flow in a unique manner. This result was not surprising given the differences in surface complexity among the four substrates. For example, Plexiglas® is very smooth. As a result, friction between flowing water and the surface is low; flow speed was minimally reduced beyond 1–2 mm away from the surface. In contrast to Plexiglas®, the various rock substrates are complex with numerous larger-scale surface irregularities. Frictional forces resulting from flowing water–rock interactions are high; flow above these obstacles was greatly reduced within a height of 2 cm.

At 17 cm s^{-1}, the boundary layer associated with the rock substrate and the region of reduced flow downstream of the large rock were similar in height (~2 cm). However, flow over the large rock was characterized by a much steeper velocity gradient than that over the small rock substrate. Because the steepness of the velocity gradient was positively correlated with the magnitude of the positive lift force that it generates, a fish or other organism that is displaced off the substrate behind a large rock will have to contend with greater upward lift forces than one that is displaced from rock substrate (Koehl, 1984). Thus, although the size of the region of reduced flow behind a large rock may be similar to that of the boundary layer on many small rocks, the hazards of displacement are expected to be greater for a fish behind a large rock than for one holding station in the open. We therefore predict that darter species that occupy riffles and runs with many large rocks (i.e. *Nothonotus* darters in high-gradient streams) (Page, 1983) will exhibit behaviors that minimize the possibility of encountering the steep velocity gradient behind these rocks. For example, individuals may edge around the sides of rocks instead of attempting to dart (and, in doing so, rise slightly off the bottom) between two rocks.

We also found that the boundary layer can exhibit unexpected features on rocky substrates. For example, we measured a significant

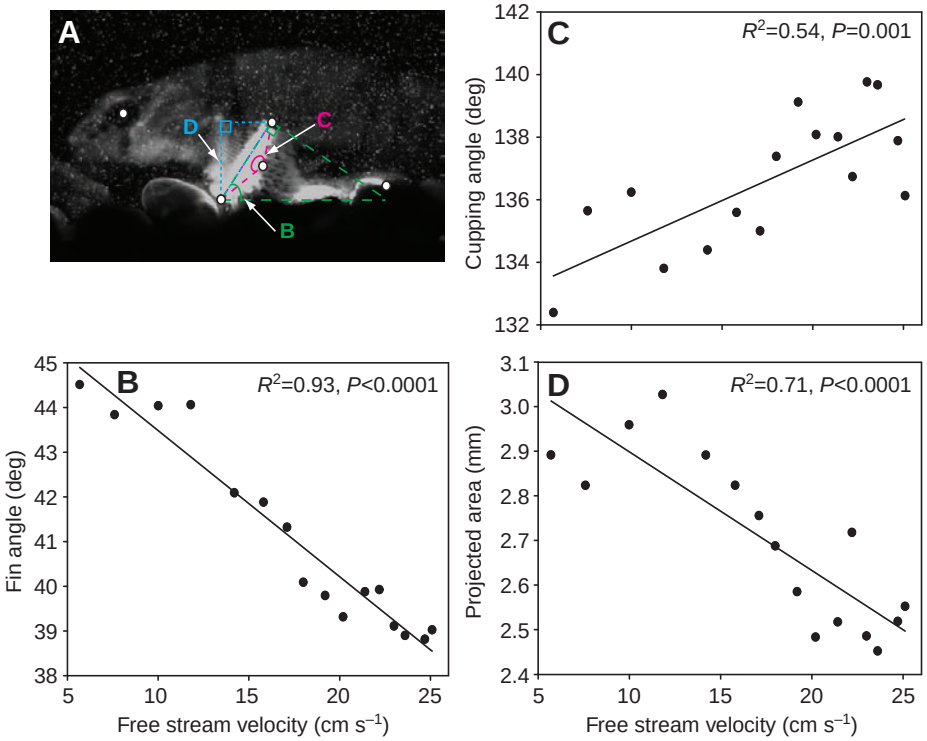

Fig. 12. Change in three pectoral fin variables with increasing free-stream flow velocity from a representative sequence of *E. tetrazonum* holding station on rocks. (A) Video still with the locations of the five digitized points as well as the angles and distance calculated from those points. Letters in A indicate the scatterplot to which each quantity corresponds: (B) change in angle of the pectoral fin relative to the horizontal, (C) extent of fin cupping and (D) projected area.

increase in boundary layer thickness (from 1.5 to 1.8 cm) as flow velocity increased from 4 to 17 cm s^{-1}. At 31 cm s^{-1}, the boundary layer decreased to a thickness of 1.5 cm in accordance with expectations. This contrasts with the canonical expectation that boundary layer thickness will always decrease with increasing flow speed and, hence, Reynolds number (Hoerner, 1965; Vogel, 1994) and suggests a complex non-textbook relationship between boundary layer flows and rough substrates that has yet to be fully explored.

Role of the pectoral fins in benthic station-holding

In darters, benthic station holding is facilitated both by living within the region of reduced flow associated with complex substrates and, to a much lesser extent, by the substrate-directed (i.e. negative lift) forces generated by the fish's own pectoral fins. Leopard and bamboo sharks, *Triakis semifasciata* and *Chiloscyllium plagiosum*, respectively, use their pectoral fins to generate negative lift forces when station holding in midwater (Wilga and Lauder, 2000; Wilga and Lauder, 2001) and, for *C. plagiosum*, when on the bottom. Our data suggest that darters use their pectoral fins in a similar manner when holding station on the bottom but that the bony fish's fins produce much weaker forces than those of the sharks.

As water flows over the pectoral fins of a station-holding darter such as *E. tetrazonum*, the flow separates and forms a series of clockwise-rotating vortices. At ~27 cm s^{-1} on rocks, this circulation generates negative lift forces equal to –0.000125 mN. The magnitude of the force is small, amounting to only ~1% of the mass of an adult *E. tetrazonum* when the fish is underwater. Thus, although the pectoral fins in this darter species do help the fish to hold station (see also Carlson and Lauder, 2010), the forces are not large. This finding is quite surprising given the large size of the pectoral fins in many darter species, including *E. tetrazonum*. We therefore posit that frictional forces between the rough substrate and the edges of the body, anal fin, and pelvic and pectoral fins are more important contributors to station-holding performance. Large, wing-like fins have relatively longer, straighter edges than round fins and would therefore provide a greater area with which to contact the substrate.

Kinematic data provide additional evidence that the pectoral fins perform some role, albeit a small one, in benthic station holding: *E. tetrazonum* exhibits a series of changes in pectoral fin orientation with increasing speed that are consistent between fish holding station on both Plexiglas® and rock substrates. Most notably, fish reduced the angle of the pectoral fin relative to the substrate and increased the amount of cupping (i.e. lower cupping angle) with increasing water velocity. The projected area of the fin was also reduced with increasing flow speed. Additionally, these changes in pectoral fin conformation suggest that the fish were attempting to minimize downstream drag forces rather than using the fins as negative lift generators.

In sum, we found that complex substrates can strongly influence the characteristics of flow and produce a 'thick' boundary layer with non-canonical characteristics. This aspect of flow modification has important consequences for station-holding darters. In particular, the velocity of the flow experienced by the fish will be reduced more by complex substrates than it is by smooth substrates such as Plexiglas®. In addition, the velocity gradient between the region of reduced flow and the free-stream flow was much steeper on Plexiglas® than on rock substrate. In combination, the relatively larger reduction in flow speed and shallower slope of the velocity gradient are expected to facilitate station holding. Our previous work with two darter species, *E. tetrazonum* and *E. flabellare*, revealed exactly this pattern: both species were able to hold station at higher flow speeds on rocks than on Plexiglas® substrates (Carlson and Lauder, 2010). Although this result could also be due to increased frictional forces between the fish and the rocks as compared with Plexiglas®, the data presented here on boundary layer thickness suggest that there is a thick zone of reduced flow on rocky substrates that extends to a height greater than that of the darter body (2 cm), and that improved station-holding performance may in fact be largely due to the greatly reduced flow velocities encountered by darters in this thick boundary layer region.

A vertebrate radiation into the boundary layer

Substrates composed of a combination of large and small rocks are characteristic of many high-gradient streams in North America (Galat et al., 2005; Jackson et al., 2005; White et al., 2005). Based on the boundary layer thickness data presented here, even in fast riffles, such substrates are expected to provide relatively thick regions of reduced flow that are large enough for all but the largest darter species. *In situ* measurements of flow speed in high-gradient streams such as the Duck River (TN, USA), the Gasconade River (MO, USA) and the French Broad River (NC, USA) are consistent with this hypothesis: there is a dramatic increase in water velocity between the substrate and the surface of the water (R.L.C., unpublished data). Notably, both the Duck and French Broad Rivers are characterized by high darter species diversity (12 and 6 species, respectively).

Occupation of lotic environments is often associated with adaptations (morphological or behavioral) that enable species to escape the brunt of the oncoming flow. Flatfishes, for example, have become increasingly thin and dorso-ventrally flattened (Arnold and Weihs, 1978). Previous researchers have argued that darters exhibit reduced body size in order to take advantage of extremely small prey such as the aquatic insect larvae on which many species feed (Page and Swofford, 1984). Our data, in addition to phylogenetic and comparative data, suggest that small body size in darters is also an adaptation to living on or near the substrate in high flow environments. Specifically, the difference in maximum body size (total length) and body depth between darters and their closest percid relatives, including walleye (total length=760 mm), yellow perch (310 mm) and sauger (460 mm), is striking. Notably, these non-darter percid species also inhabit mostly lentic, or non-flowing, environments (Page and Burr, 1991; Page and Swofford, 1984). In contrast, species of darters average 70–80 mm in standard length and 19 mm in body depth (Carlson and Wainwright, 2010; Page, 1981; Page and Swofford, 1984). Phylogenetic evidence also suggests that the evolution of small body size occurred concurrently with the invasion of lotic habitats and the acquisition of a benthic lifestyle, lending credence to the hypothesis that, in darters, small size is associated with use of the benthic boundary layer (Song et al., 1998).

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REFERENCES

Allan, J. D. (1995). *Stream Ecology: Structure and Function of Running Waters.* Dordrecht, The Netherlands: Kluwer Academic Publishers.
Anderson, E. J., McGillis, W. and Grosenbaugh, M. A. (2001). The boundary layer of swimming fish. *J. Exp. Biol.* **204**, 81-102.
Arnold, G. P. and Weihs, D. (1978). The hydrodynamics of rheotaxis in the plaice (*Pleuronectes platessa* L.). *J. Exp. Biol.* **75**, 147-169.
Blake, R. W. (2006). Biomechanics of rheotaxis in six teleost genera. *Can. J. Zool.* **84**, 1173-1186.

Brooks, A. J., Haeusler, T., Reinfelds, I. and Williams, S. (2005). Hydraulic microhabitats and the distribution of macroinvertebrate assemblages in riffles. *Freshw. Biol.* **50**, 331-344.
Carlson, R. L. (2008). The evolution of ecological and morphological diversity in darter fishes (Percidae: Etheostomatinae). PhD thesis, University of California, Davis, CA, USA.
Carlson, R. L. and Lauder, G. V. (2010). Living on the bottom: kinematics of benthic station-holding in darter fishes (Percidae: Etheostomatinae). *J. Morphol.* **271**, 25-35.
Carlson, R. L. and Wainwright, P. C. (2010). The ecological morphology of darter fishes (Percidae: Etheostomatinae). *Biol. J. Linn. Soc.* **100**, 30-45.
Cotel, A. J., Webb, P. W. and Tritico, H. M. (2006). Do brown trout choose locations with reduced turbulence? *Trans. Am. Fish. Soc.* **135**, 610-619.
Denny, M. W. (1993). *Air and Water: The Biology and Physics of Life's Media.* Princeton, NJ: Princeton University Press.
Dodds, G. S. and Hisau, F. L. (1924). Ecological studies of aquatic insects. I. Adaptations of mayfly nymphs to swift streams. *Ecology* **5**, 137-148.
Drucker, E. G. and Lauder, G. V. (1999). Locomotor forces on a swimming fish: three-dimensional vortex wake dynamics quantified using digital particle image velocimetry. *J. Exp. Biol.* **202**, 2393-2412.
Galat, D. L., Berry, C. R., Peters, E. J. and White, R. G. (2005). Missouri river basin. In *Rivers of North America* (ed. A. C. Benke and C. E. Cushing), pp. 427-480. New York: Elsevier Academic Press.
Hart, D. D. and Finelli, C. M. (1999). Physical–biological coupling in streams: the pervasive effects of flow on benthic organisms. *Annu. Rev. Ecol. Syst.* **30**, 363-395.
Hedrick, T. L. (2008). Software techniques for two- and three-dimensional kinematic measurements of biological and biomimetic systems. *Bioinspir. Biomim.* **3**, 034001.
Helfman, G. S., Collette, B. B. and Facey, D. E. (1999). *The Diversity of Fishes.* Malden, MA: Blackwell Science, Inc.
Hoerner, S. F. (1965). *Fluid-Dynamic Drag.* Bakersfield, CA: Hoerner Fluid Dynamics.
Jackson, J. K., Huryn, A. D., Strayer, D. L., Courtemanch, D. L. and Sweeney, B. W. (2005). Atlantic Coast rivers of the Northeastern United States. In *Rivers of North America* (ed. A. C. Benke and C. E. Cushing), pp. 21-71. New York: Elsevier Academic Press.
Koehl, M. A. R. (1984). How do benthic organisms withstand moving water? *Am. Zool.* **24**, 57-70.
Lancaster, J. and Hildrew, A. G. (1993). Flow refugia and the microdistribution of lotic macroinvertebrates. *J. N. Am. Benthol. Soc.* **12**, 385-393.
Lauder, G. V. and Madden, P. G. (2008). Advances in comparative physiology from high-speed imaging of animal and fluid motion. *Annu. Rev. Physiol.* **70**, 8.1-8.21.
MacDonnell, A. J. and Blake, R. W. (1990). Rheotaxis in *Otocinclus* sp. (Teleostei: Loricariidae). *Can. J. Zool.* **68**, 599-601.
McCune, A. R. and Carlson, R. L. (2004). Twenty ways to lose your bladder: common natural mutants in zebrafish and widespread convergence of swim bladder loss among teleost fishes. *Evol. Dev.* **6**, 246-259.
Nelson, J. S. (1994). *Fishes of the World*, 3rd edn. New York: John Wiley and Sons, Inc.
Page, L. M. (1981). The genera and subgenera of darters (Percidae, Etheostomatini). *Occas. Pap. Mus. Nat. Hist. Univ. Kansas* **90**, 1-69.
Page, L. M. (1983). *Handbook of Darters.* Neptune City, NJ: T.F.H. Publications, Inc.
Page, L. M. and Burr, B. M. (1991). *A Field Guide to Freshwater Fishes: North America North of Mexico.* New York: Houghton Mifflin Company.
Page, L. M. and Swofford, D. L. (1984). Morphological correlates of ecological specialization in darters. *Environ. Biol. Fishes* **11**, 139-159.
Pearson, R. G. and Boyero, L. (2009). Gradients in regional diversity of freshwater taxa. *J. N. Am. Benthol. Soc.* **28**, 504-514.
Power, M. E., Sout, R. J., Cushing, C. E., Harper, P. P., Hauer, F. R., Matthews, W. J., Moyle, P. B., Statzner, B. and Wais De Badgen, I. R. (1988). Biotic and abiotic controls in river and stream communities. *J. N. Am. Benthol. Soc.* **7**, 456-479.
Prandtl, L. and Tietjens, O. G. (1934). *Applied Hydro- and Aeromechanics.* New York: Dover Publications.
Rahman, S. and Webster, D. R. (2005). The effect of bed roughness on scalar fluctuations in turbulent boundary layers. *Exp. Fluids* **38**, 372-384.
Schlichting, H. (1979). *Boundary-Layer Theory.* New York: McGraw-Hill.
Silvester, N. R. and Sleigh, M. A. (1985). The forces on microorganisms at surfaces in flowing water. *Freshw. Biol.* **15**, 433-448.
Song, C. B., Near, T. J. and Page, L. M. (1998). Phylogenetic relations among percid fishes as inferred from mitochondrial cytochrome *b* DNA sequence data. *Mol. Phylogenet. Evol.* **10**, 343-353.
Standen, E. M. and Lauder, G. V. (2007). Hydrodynamic function of dorsal and anal fins in brook trout (*Salvelinus fontinalis*). *J. Exp. Biol.* **210**, 325-339.
Statzner, B. and Holm, T. F. (1982). Morphological adaptations of benthic invertebrates to stream flow-an old question studied by means of a new technique (laser Doppler anemometry). *Oecologia* **53**, 290-292.
Statzner, B. and Holm, T. F. (1989). Morphological adaptation of shape to flow: microcurrents around lotic macroinvertebrates with known Reynolds numbers at quasi-natural flow conditions. *Oecologia* **78**, 145-157.
Tytell, E. D. and Lauder, G. V. (2004). The hydrodynamics of eel swimming. I. Wake structure. *J. Exp. Biol.* **207**, 1825-1841.
Vogel, S. (1994). *Life in Moving Fluids: The Physical Biology of Flow.* Princeton, NJ: Princeton University Press.
Webb, P. W. (1989). Station-holding by three species of benthic fishes. *J. Exp. Biol.* **145**, 303-320.
Webb, P. W., Gerstner, C. L. and Minton, S. T. (1996). Station-holding by the mottled sculpin, *Cottus bairdi* (Teleostei: Cottidae), and other fishes. *Copeia* **3**, 488-493.
White, D., Johnston, K. and Miller, M. (2005). Ohio river basin. In *Rivers of North America* (ed. A. C. Benke and C. E. Cushing). New York: Elsevier Academic Press.
Wilga, C. D. and Lauder, G. V. (2000). Three-dimensional kinematics and wake structure of the pectoral fins during locomotion in leopard sharks *Triakis semifasciata*. *J. Exp. Biol.* **203**, 2261-2278.
Wilga, C. D. and Lauder, G. V. (2001). Functional morphology of the pectoral fins in bamboo sharks, *Chiloscyllium plagiosum*: benthic versus pelagic station holding. *J. Morphol.* **249**, 195-209.